

Immunization with Purified Protein Antigens from *Streptococcus mutans* Against Dental Caries in Rhesus Monkeys

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Protein antigens I, I/II, II, and III were prepared from *Streptococcus mutans* (serotype c). Their immunogenicities and protective effects against dental caries were investigated in 40 rhesus monkeys kept entirely on a human-type diet, containing about 15% sucrose. Antigens I, I/II and, to a lesser extent, antigen II induced significant reductions in dental caries, as compared with sham-immunized monkeys. This was achieved with 1 or 2 doses of antigen, the first of which was administered with adjuvant (Freund incomplete adjuvant or aluminum hydroxide). There was no reduction in caries in monkeys immunized with antigen III. The reduction in caries in the animals immunized with antigens I or I/II was comparable to that in monkeys immunized with whole cells. Protection against caries was associated predominantly with serum and gingival crevicular fluid immunoglobulin G antibodies, which appeared to be directed against the antigen I determinant, but antibodies to antigen II, though not to antigen III, were also protective.

Immunization against dental caries has recently utilized specific components isolated from *Streptococcus mutans*. Glucosyl transferase fractions were prepared from *S. mutans* and used successfully in the immunization of rats and hamsters (24). We have detected and purified three protein antigens from *S. mutans* (15, 17, 18), one of which induced significant protection against caries when injected into rhesus monkeys (11). The three antigens, which can be more efficiently isolated from culture fluids, have been designated as antigens I, II, and III, according to their electrophoretic mobility (17). Antigens I and II appear to be two determinants present in a single molecule, which has been purified and termed antigen I/II (15). Antigen I/II has a molecular weight of 185,000, and this agrees reasonably well with the sum of the molecular weights of separately purified antigen I (150,000) and antigen II (48,000), as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antigen III has a molecular weight of approximately 40,000 (14).

A functional difference has been established between antigens I, II, and III, in that rhesus monkeys immunized with cells or cell walls of *S. mutans* and protected from dental caries develop antibodies to antigens I and II but weak or no antibodies to antigen III (16). The protective properties of antigen I/II against dental

caries have been examined directly by immunizing rhesus monkeys subcutaneously with 1 mg of antigen I/II in Freund incomplete adjuvant (FIA) or aluminum hydroxide (11). The results suggested that antigen I/II was as effective as whole cells of *S. mutans* (serotype c), from which the antigen had been prepared, in significantly reducing the development of dental caries. Protection was related to serum immunoglobulin G (IgG) antibodies, which increased very significantly in the immunized, as compared to the sham-immunized, monkeys. The IgG antibody fluorescence titer of 1:640 was well maintained, after a single episode of immunization, throughout the experiment, which lasted up to 92 weeks.

The aims of the present investigation were to examine the protective function of purified antigens I, I/II, II, and III in rhesus monkeys and to study the class and specificity of serum and salivary antibodies, as well as the changes in colonization of teeth by *S. mutans*.

MATERIALS AND METHODS

Preparation of antigens. *S. mutans* (serotype c, Guy's strain) was isolated from the dental plaque of a child with a high prevalence of caries. All antigens were prepared from this strain of *S. mutans*. For immunization with the cells, the organisms were grown in Todd-Hewitt broth and killed with 0.6% Formalin, and a suspension containing 10^9 cells per ml was prepared (8).

Antigen I/II was prepared from the culture supernatant of *S. mutans* grown in a semidefined medium (1), as described previously (15, 17). Briefly, the culture supernatant was precipitated by ammonium sulfate and chromatographed on diethylaminoethyl (DEAE)-cellulose to yield partially purified antigen I/IIa. Purified antigen I/IIb was prepared in the same way as antigen I/IIa, with the further purification step of gel filtration on Sepharose 6B (15).

Antigen Ia (partially purified) was prepared in a similar manner to antigen I/IIa, but was eluted from DEAE-cellulose before antigen I/II with the starting buffer and was further chromatographed on Sepharose 6B (18). Purified antigen Ib was prepared as antigen Ia, except that the material eluted from DEAE-cellulose was affinity-purified on tandem immunoadsorbent columns (18).

Antigen IIa (partially purified) was prepared from antigen I/IIa by pronase treatment followed by gel filtration on Bio-Gel P150 (15). The preparation of purified antigen IIb was similar to that of antigen IIa, except that the pronase-treated material was separated by gel filtration on Ultrogel AcA 34 (15).

Antigen III was eluted from DEAE-cellulose at higher ionic strength than antigen I/II, to give partially purified antigen IIIa. This was further purified by repeated chromatography on DEAE-cellulose and gel filtration on Ultrogel AcA 34 to give purified antigen IIIb (14).

Animals. Young rhesus monkeys weighing between 1.4 and 2.2 kg were selected for the presence of all their deciduous teeth; in a few the first permanent molars were erupting. Forty monkeys were caged, examined, and kept entirely on a human-type diet within 5 days of starting the experiment (8). The animals were divided randomly into six groups (Table 1). Five monkeys were each injected subcutaneously with 5×10^8 Formalin-killed cells of *S. mutans* (serotype c, Guy's strain) in FIA, in equally divided doses, into an upper and lower limb. Five monkeys were injected with antigen I/II: two monkeys were each given 10 mg of antigen I/IIa in FIA subcutaneously, and three monkeys were given 1 mg of antigen I/IIb, two with FIA and one with Alhydrogel (Miles Laboratories Ltd.), into the same two sites. Five monkeys

were similarly given antigen I: three monkeys had 0.5 mg of antigen Ia in FIA, and two monkeys had 0.1 mg of antigen Ib in Alhydrogel. A group of seven monkeys was immunized with antigen II: five monkeys were given 1 to 2 mg of antigen IIa with FIA (except one with Alhydrogel), and two monkeys had 0.1 mg of antigen IIb with Alhydrogel as above. Antigen III was administered to four monkeys: two monkeys were each given 10 mg of antigen IIIa with FIA and two monkeys had 1 mg of antigen IIIb with Alhydrogel. Furthermore, 14 monkeys were sham-immunized with saline.

A second dose of antigen was given to all of the cell-immunized monkeys 16 to 28 weeks after primary immunization, using the same number of cells but no adjuvant. Secondary immunization in the other groups was carried out only in the monkeys injected with the early preparations of antigens, i.e., I/IIa, IIa, and IIIa. They were injected at 16 to 24 weeks with the same antigen, but the dose was kept to 1 to 2 mg, and no adjuvant was used. The monkeys immunized with antigens Ia, Ib, I/IIb, and IIIb had no secondary immunization. The duration of the experiments was 72 or 92 weeks, and this was always balanced between the immunized and control animals. The results of three of the cell-immunized, three of the antigen I/II-immunized, and four of the sham-immunized monkeys were published before (11).

Dental caries. Dental caries was assessed conventionally by examination with a mirror and probe and radiologically at monthly intervals, as described before (8). Caries was recorded by a caries score, which is the mean of the number of smooth-surface and fissure cavities in the deciduous teeth and first permanent molars in each group of monkeys (7).

Antibodies. Blood was collected from the femoral vessels, and the serum was separated. Serum IgG, IgA, and IgM antibody titers were determined by the indirect immunofluorescent method against air-dried smears of *S. mutans* (12). Specific rabbit antihuman IgG fluorescein conjugates (F:P 3:1; Wellcome Laboratories), anti-rhesus monkey IgA (F:P 1:4; Nordic Laboratories), and IgM (Nordic Laboratories) which was conjugated with fluorescein isothiocyanate (5) (F:P 5:1) were used. The specificities of these conjugates were determined as described before (10).

TABLE 1. Immunization schedule in six groups of rhesus monkeys

Primary immunization				Secondary immunization			
Antigen group	No. of monkeys	Antigen prepn	Dose/adjuvant	No.	Dose	No.	Time (wk) ^a
Cells I/II	5	Cells	5×10^8 /FIA	5	5×10^8	5	16-28
	5	I/IIa	10 mg/FIA	2	2 mg	2	24
		I/IIb	1 mg/FIA ^b	3	— ^c	—	—
I	5	Ia	0.5 mg/FIA	3	—	—	—
		Ib	0.1 mg/Al(OH) ₃	2	—	—	—
II	7	IIa	1-2 mg/FIA ^b	5	1-2 mg	5	16-24
		IIb	0.1 mg/Al(OH) ₃	2	—	—	—
III	4	IIIa	10 mg/FIA	2	2 mg	2	16
		IIIb	1 mg/Al(OH) ₃	2	—	—	—
Control	14	Saline	1 ml	14	1 ml	14	16-28

^a Interval between primary and secondary immunization.

^b One of the monkeys had Al(OH)₃.

^c —, Not done.

A radioimmunoassay for serum antibodies to antigen I, II, I/II, and III was used as described elsewhere (20). Briefly, polystyrene tubes were coated with 10 μ g of antigen I, 2 μ g of antigen II, 1 μ g of antigen I/II, and 1 μ g of antigen III (dry weight) per ml. The tubes were then treated with 0.5% bovine serum albumin, phosphate-buffered saline and 0.05% Tween 20, washed, and incubated in duplicate with serially diluted monkey sera. After three washes, the tubes were incubated with 125 I-labeled rabbit anti-monkey IgG (Fc) at a concentration of 1 μ g/ml. The bound 125 I-labeled anti-IgG was assessed in a Beckman gamma counter. The results were expressed as the percentage of bound radioactivity. Controls included in each experiment were antigen-coated tubes without added serum or with reference immune and control sera. The specificity of the assay had been tested previously by competitive inhibition (20).

The sensitivity of the above double-layer radioimmunoassay was increased 10 times by a triple antibody radioimmunoassay which enabled antibodies in gingival crevicular washings to be examined (20). The method was as described above, except that the rabbit anti-monkey IgG was used at 10 μ g per ml and was not radiolabeled and that, after washing, the tubes were treated with 125 I-labeled swine anti-rabbit IgG at a concentration of 1 μ g/ml. Gingival crevicular fluid was collected by the washing method (21).

Oral fluid was collected by allowing saliva to flow into a petri dish after injection of 0.5 mg of pilocarpine per kg subcutaneously. Antibodies in oral fluids were determined by direct agglutination of streptococcal cells (3). Antibodies in the oral fluid were characterized for the IgA class specificity elsewhere (3).

Culture of *S. mutans*. Plaque was collected with sterile probes from the cervical and approximal surfaces of the upper-left deciduous molars and from the fissures of the adjacent first permanent molar. The samples were placed into transport medium and grown on tryptone-yeast extract L-cystine (TYC) medium (22). The number of colony-forming units of *S. mutans* was determined as described previously (2) and expressed as a percentage of the total colony count on TYC medium. In view of the known variability in the estimation of colony-forming units, the results are expressed in terms of the cumulative mean of monthly assays of colony-forming units over the first 12, 20, and 28 weeks. After 28 weeks the colony-forming units were estimated at about two monthly intervals, and

again the cumulative mean was calculated at 40 and 72 weeks.

RESULTS

The mean (\pm standard error) of the caries index of monkeys immunized with antigens I, I/II, and II was 5 (± 1.0), as compared with those of the sham-immunized group (11.0 ± 1.8), 72 weeks after the experiment was started (Table 2). Analysis by Student's *t* test showed a very significant difference in the caries index between these two groups ($t = 3.0600$, df 29, $P < 0.01$). This was comparable with the reduction in caries reached by the cell-immunized monkeys ($t = 2.1303$, df 17, $P < 0.05$).

Although the monkeys were immunized with two different preparations and doses of each of the four protein antigens, the caries indices between the partially purified "a" and later purified "b" preparations of the same antigen were comparable. The results are therefore presented for the whole group (Table 2), and they are then also amenable to statistical analysis. Significant reductions in the caries indices were found in antigen I/II- ($t = 2.181$, df 17, $P < 0.05$) and antigen I- ($t = 2.140$, df 17, $P < 0.05$) immunized groups, when compared with the sham-immunized monkeys (Table 2). The antigen II-immunized monkeys also showed a lower mean caries index (6.4 ± 1.7) than the controls, but the 5% level of significance was not reached ($t = 1.691$, df 19). These results were maintained when analyzed at 92 weeks (Table 2) with smaller groups of monkeys. However, the reduction in caries in the antigen II-immunized monkeys now also reached the 5% level of significance ($t = 2.344$, df 11). The antigen III-immunized monkeys failed to show a significant reduction in smooth-surface or fissure caries. Sequential development of caries (Fig. 1) illustrates the lack of protection in monkeys immunized with antigen III, the intermediate position in those immunized with antigen II, and the protection in monkeys immunized with antigens I, I/II, or cells.

TABLE 2. Caries indices in six groups of monkeys

Immunizing antigen	72 wk				92 wk			
	No. of monkeys	Caries index ^a	Smooth surface ^a	Fissure ^a	No. of monkeys	Caries index ^a	Smooth surface ^a	Fissure ^a
Cells	5	4.4 (1.3)	3.2 (1.2)	1.2 (0.2)	5	5.8 (2.0)	4.0 (1.8)	1.8 (0.4)
I/II	5	4.2 (1.5)	2.6 (0.8)	1.6 (0.8)	5	5.2 (1.9)	2.8 (1.0)	2.4 (1.0)
I	5	4.0 (2.3)	2.8 (1.7)	1.2 (0.6)	3	3.3 (2.0)	1.7 (1.2)	1.7 (0.9)
II	7	6.3 (1.7)	5.0 (1.2)	1.3 (0.7)	4	4.2 (1.6)	4.0 (1.5)	0.2 (0.2)
III	4	10.2 (2.5)	7.7 (1.5)	2.5 (1.0)				
Saline	14	11.0 (1.8)	6.7 (1.5)	4.3 (0.8)	9	13.2 (2.4)	7.8 (2.3)	5.4 (1.0)
I/II, I, II	17	5.0 (1.0)	3.6 (0.7)	1.4 (0.4)				

^a Mean (\pm standard error).

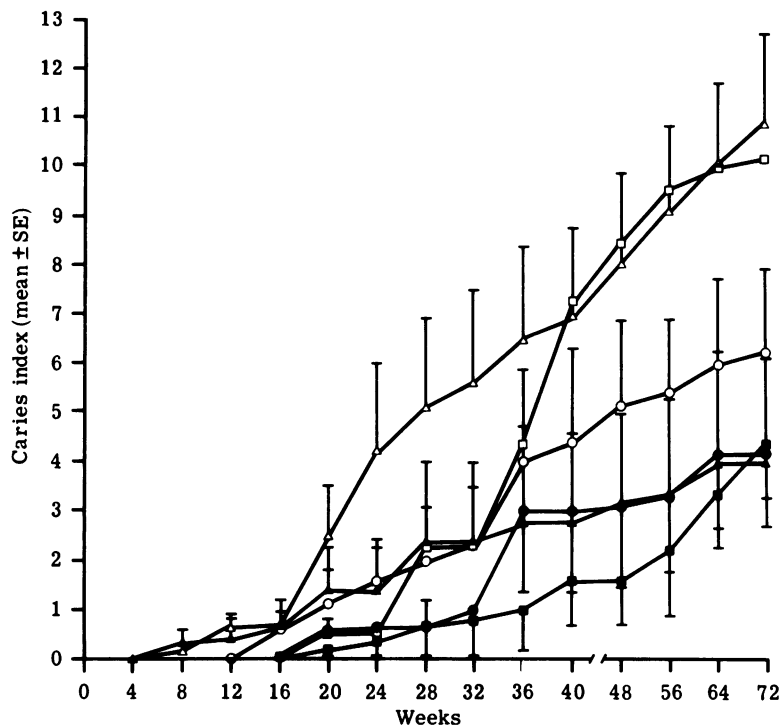


FIG. 1. Sequential development of caries in monkeys sham immunized (Δ) or immunized with antigens I/II (\bullet), I (\blacktriangle), II (\circ), III (\square), and cells (\blacksquare).

Separation of caries into the smooth-surface and fissure types showed that at 72 weeks there was a comparable, though slightly greater, reduction in fissure than in smooth-surface caries in monkeys immunized with cells, antigen I/II, or I (Table 2). At 92 weeks, however, antigen I/II- or I-immunized monkeys showed a slightly greater reduction in smooth-surface than in fissure caries. Only antigen II induced a consistently greater reduction in fissure than in smooth-surface caries.

Serum antibodies to cells. All of the antigen- and whole-cell-immunized monkeys developed IgG antibodies with a \log_2 titer of 5 to 8 by 8 weeks, as compared with the sham-immunized monkeys which had a \log_2 titer of less than 1 (Fig. 2). The highest titers were reached by the antigen I/II-immunized monkeys, and the lowest titers were reached by those immunized with antigen II. The antibody levels were well maintained in all groups of monkeys, including those which had not received a second immunizing dose (antigen groups I, I/IIb, IIIb). The high titers in the antigen III group were due to lack of purity in the antigen IIIa preparation, which was injected at a high dose of 10 mg; the mean \log_2 titer for the IIIa-immunized monkeys was 8.5 as compared with 3 for the IIIb-immunized monkeys.

Serum IgA antibodies reached lower titers than did IgG antibodies (Fig. 3). By 8 weeks, the monkeys immunized with antigen I showed titers of \log_2 2.8, whereas in the three other antigen-immunized groups, the titers were between \log_2 0.7 and 1.6. There was a further increase in the antibody level up to week 20 to 28, and the titers were then well maintained.

Serum IgM antibodies also reached lower titers than did IgG antibodies (Table 3). By about 8 weeks, monkeys immunized with cells showed titers of \log_2 6.0 (± 0.55), and this was progressively decreased in the groups immunized with antigen I/II, III, I and II.

Serum antibodies to antigens I, II, I/II, and III. IgG antibodies to the four defined antigens examined by radioimmunoassay of sera 8 to 12 weeks after immunization showed clearly that the highest antibody titers against any one of these antigens were elicited by the homologous antigen (Fig. 4). Immunization with whole cells elicited antibody levels to each antigen comparable with those induced by the homologous antigen. As with the fluorescent-antibody assay, negligible antibody binding was found in the sham-immunized monkeys, except to antigen I/II; the latter (0.97 ± 0.32) was similar to that found in the preimmunized sera (0.74 ± 0.27). Antibodies to antigens I, I/II and, to a

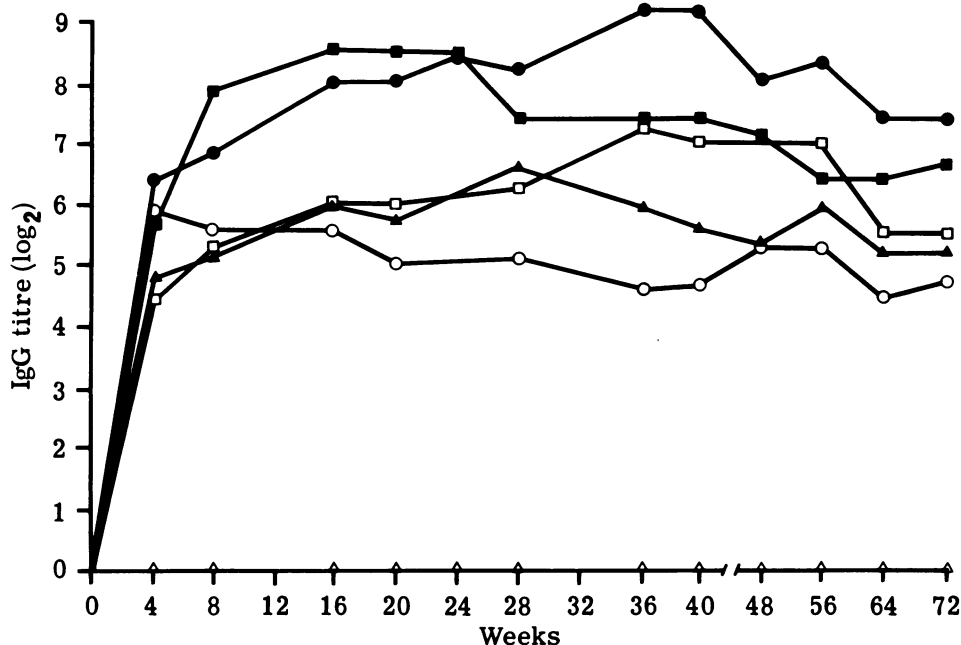


FIG. 2. Sequential development of serum IgG immunofluorescence antibodies to *S. mutans* in monkeys sham immunized (Δ) or immunized with antigen I/II (\bullet), I (\blacktriangle), II (\circ), III (\square), and cells (\blacksquare); given in \log_2 ($\log_2 1 = 1:5$, $\log_2 2 = 1:10$, etc).

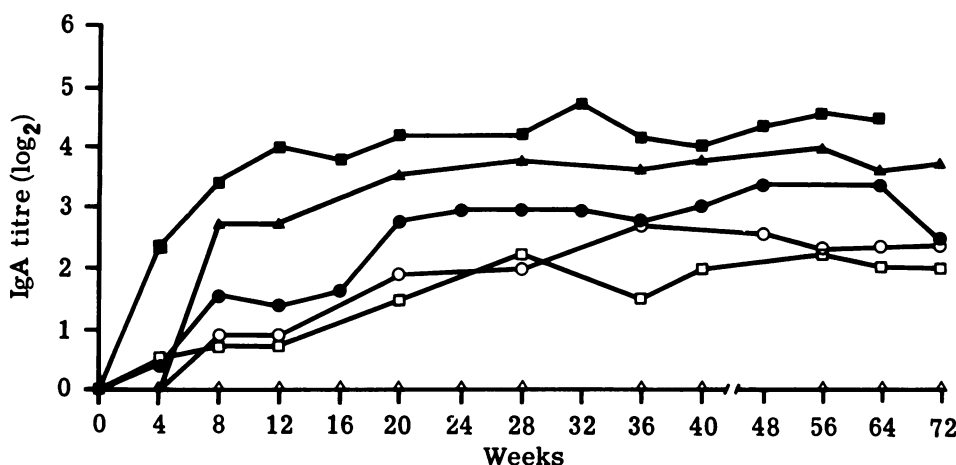


FIG. 3. Sequential development of serum IgA immunofluorescence antibodies to *S. mutans* in monkeys sham immunized (Δ) or immunized with antigen I/II (\bullet), I (\blacktriangle), II (\circ), III (\square), and cells (\blacksquare); given in \log_2 ($\log_2 1 = 1:5$, $\log_2 2 = 1:10$, etc).

lesser extent, II showed the best correlation with protection against caries, but no correlation was found with antibodies to antigen III (Fig. 4).

Gingival crevicular fluid antibodies to antigen I/II. The results with the crevicular fluids which were collected towards the end of the experiment (72 or 92 weeks) were comparable with those of the corresponding sera (Table 4). The highest radioactivity bound to antigen

I/II was shown by crevicular fluids from antigen I- ($0.35 \pm 0.12\%$) and I/II- ($0.34 \pm 0.05\%$), followed by antigen II- (0.13%) and then antigen III- (0.04%) immunized monkeys. Sham-immunized monkeys showed no significant bound radioactivity ($0.02 \pm 0.018\%$).

Salivary agglutinating antibodies. All groups of immunized monkeys showed an increase in mean salivary antibody titers by 12

weeks of \log_2 1.4 to 2.5, but the sham-immunized monkeys also showed a corresponding increase of \log_2 1.8 (Fig. 5). The maximal increase in salivary antibodies throughout the experimental period was recorded in monkeys immunized with antigen III.

Recovery of *S. mutans*. The cumulative mean recovery of *S. mutans* from plaque samples is shown in Fig. 6. The sham-immunized group of monkeys showed consistently higher

recovery of *S. mutans* than the immunized groups, at each of the time intervals up to 72 weeks. Only in the antigen III-immunized monkeys did the level of *S. mutans* colonization approach that found in the sham-immunized monkeys after week 28. The cell-immunized monkeys showed the lowest recovery of *S. mutans* of all of the immunized monkeys.

DISCUSSION

The purpose in isolating the four protein antigenic preparations from *S. mutans* (15, 17, 18) was to study their immunogenicity, in particular, their ability to induce protection against dental caries. Antigens I, I/II, and II induced protection when tested in a combined group of 17 monkeys and compared with 14 controls ($t = 3.060$, $df\ 29$, $P < 0.01$). Further analysis of the three separate groups of antigen-immunized monkeys showed that protection consistently resulted from immunization with antigens I and I/II, but only after 92 weeks with antigen II (Table 2). Surprisingly, antigen II induced 95% reduction in

TABLE 3. Mean \log_2 IgM antibodies in six groups of monkeys

Immunizing antigen	Log ₂ titers, mean (\pm SE) ^a	
	Wk 0	Wk 8
Cells	0	6 (\pm 0.55)
I/II	0	4.0 (\pm 0.55)
I	0	2.6 (\pm 0.37)
II	0	2.0 (\pm 0.82)
III	0	3.25 (\pm 1.25)
Saline	0	0

^a SE, Standard error.

SERUM IgG ANTIBODIES TO 4 PROTEIN ANTIGENS DETERMINED BY RADIOIMMUNOASSAY IN 6 GROUPS OF MONKEYS

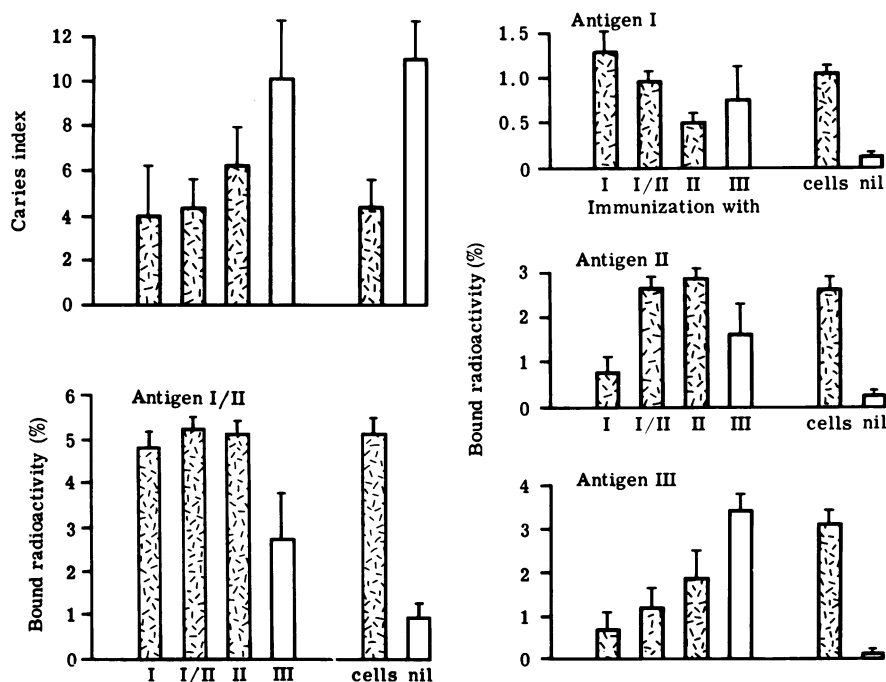


FIG. 4. Mean (\pm standard error) percent of serum bound radioactivity to streptococcal antigens I, II, I/II, and III in six groups of monkeys immunized with these antigens, cells, and saline; the sera were tested at a dilution of 1:50 and were 8 to 12 weeks post-immunization. The mean (\pm standard error) caries indices are also given for comparison.

fissure caries but only 49% in smooth-surface caries. It should be noted that whereas reduction in caries included both smooth-surface and fissure caries, there was a slightly greater reduction in fissure caries (70 to 72%) than in smooth-surface caries (58 to 61%) in monkeys immunized with antigens I and I/II. As fissure caries is more frequent in children than smooth-surface caries, the protective effect of antigens I, I/II, and II might prove to be particularly helpful. Antigen III failed to induce significant protection.

All of the immunized monkeys showed a brisk increase in serum IgG antibodies within 4 weeks of immunization, and no significant antibodies were detectable by immunofluorescence in the sham-immunized monkeys (Fig. 2). The antibody levels were well maintained throughout the experimental period; the lowest levels were in monkeys immunized with antigen II. Although antigen III yielded moderately high levels of antibodies, this was almost certainly

due to the high dose of administered antigen (10 mg). Serum IgA titers were comparable to the IgG titers, but at a much lower level. IgM antibodies about 8 weeks after immunization showed titers comparable with IgG titers, though at a lower level. The IgM titers reached levels between those of IgG and IgA in all groups of animals, except in the antigen I group. Our inability to detect IgM antibodies in the antigen I/II-immunized monkeys in an earlier study (11) proved to be due to poor cross-reactivity between the human anti-IgM antiserum and monkey IgM.

Radioimmunoassay for serum IgG antibodies specific for the four defined antigens showed that these antigens induced a predominantly homologous antibody response. Thus, immunization with antigen I induced antibodies predominantly to I (and I/II); immunization with antigen II induced antibodies predominantly to II (and I/II). As expected, antigen I/II elicited antibodies both to antigen I and II, although the response to II was stronger than to I. As the ratio of antigenic determinants I to II in the I/II complex and its tertiary structure are unknown, the relative immune response to the two determinants cannot be predicted. It is, however, reasonably clear that protection is predominantly related to antibodies to antigen I and that antibodies to antigen II, but not to III, can also be protective. These results are consistent with the opsonizing effect of antibodies in phagocytosis of *S. mutans* by polymorphonuclear leu-

TABLE 4. *Gingival crevicular fluid antibodies to antigen I/II*

Immunizing antigen	No. of monkeys tested	Bound radioactivity, mean (\pm SE) ^a %
Saline	8	0.02 (\pm 0.018)
I/II	3	0.34 (\pm 0.05)
I	5	0.35 (\pm 0.12)
II	2	0.13
III	2	0.04

^a SE, Standard error.

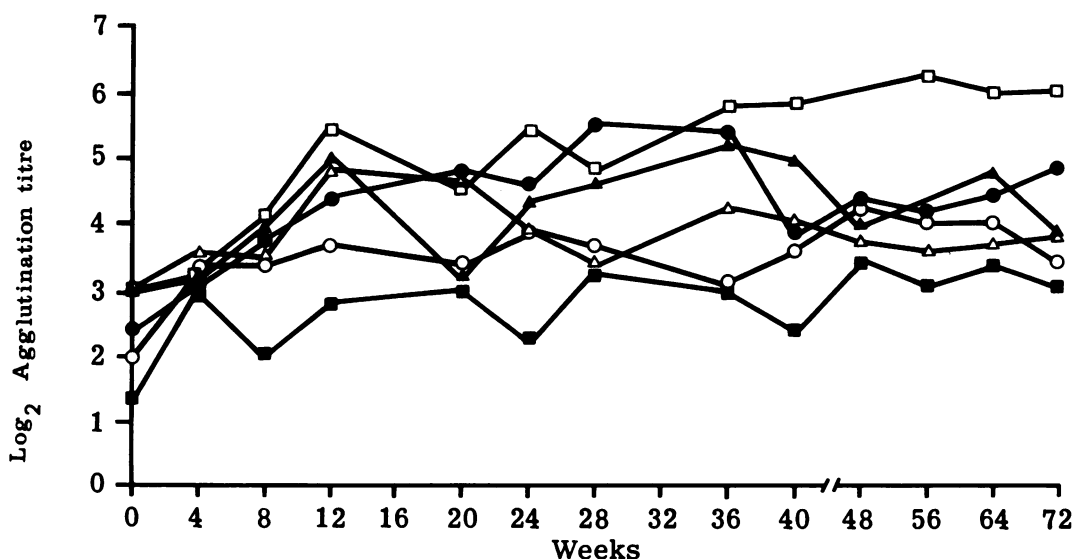


FIG. 5. Sequential development of salivary agglutinating antibodies to *S. mutans* in monkeys sham immunized (Δ) or immunized with antigen I/II (\bullet), I (\blacktriangle), II (\circ), III (\square), and cells (\blacksquare); given in \log_2 ($\log_2 1 = 1:2$, $\log_2 2 = 1:4$, etc.).

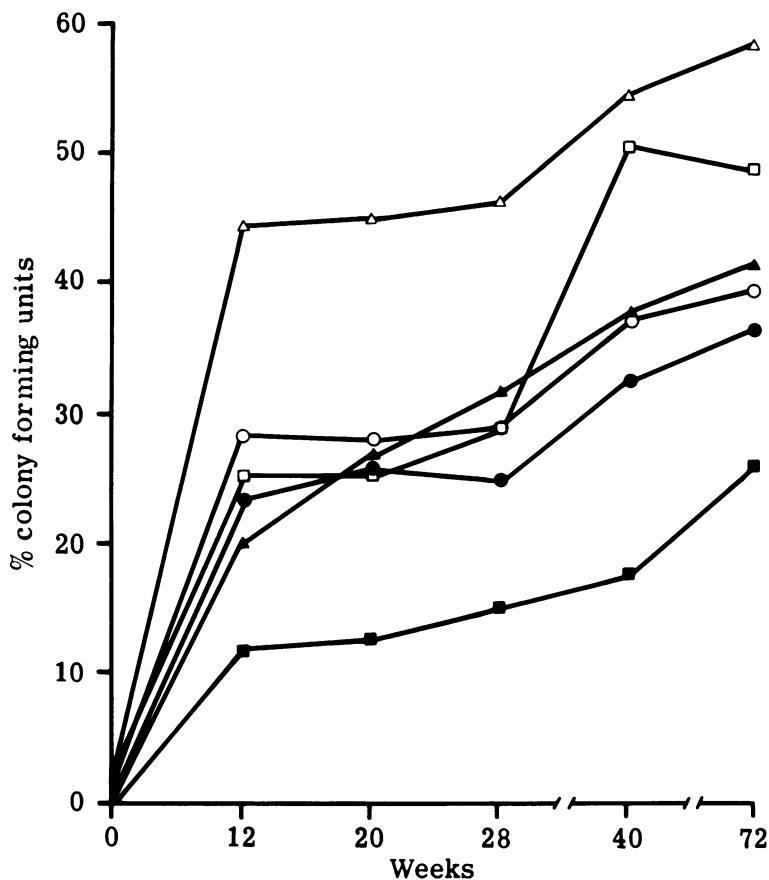


FIG. 6. Cumulative mean recovery of *S. mutans* (as percentage of total recovered bacterial count) in monkeys sham-immunized (Δ), or immunized with antigen I/II (\bullet), I (\blacktriangle), II (\circ), III (\square), and cells (\blacksquare).

kocytes. Opsonization was observed with antibodies I, I/II, and II, but not with antibodies to antigen III (19). These results also indicate the importance of antigen I and II in the interaction between *S. mutans* and the host immune response.

The passage of immunoglobulins from serum to crevicular fluid has been established in the rhesus monkey (4). Antibodies to antigen I/II have not been detected directly in gingival crevicular fluid, and a significant correlation has been established between serum and crevicular fluid antibodies to antigen I/II (20). In the present work, IgG antibodies to antigen I/II were found in both serum and crevicular fluid and were associated with protection against dental caries. Thus, the highest levels of antibodies were found in crevicular fluid of monkeys immunized with antigens I and I/II (with best protection against caries), lower levels were found in antigen II-immunized monkeys (with less protection), and very low levels were found in antigen III-immunized monkeys, which

showed no protection. These results are consistent with the hypothesis that serum IgG antibodies pass from the circulation into gingival crevicular fluid, from where they enter the oral fluid, so that they may function in both the crevicular and salivary domains of the tooth.

Salivary agglutinating antibody titers were slightly increased in all immunized groups and also in sham-immunized monkeys (Fig. 5). No protective relationship could be established between salivary antibodies and dental caries in rhesus monkeys after immunization with protein antigens or cells of *S. mutans*. This is consistent with previous attempts which failed to correlate salivary IgA antibodies with protection against dental caries in rhesus monkeys, with different antigenic preparations, administered by subcutaneous, submucosal, or oral routes (3, 9). These results, however, are at variance with the experience in immunization of rats by repeated paravascular injections (23, 24) or by the oral route (13).

Immunized monkeys showed a consistently

lower mean level of *S. mutans* in dental plaque samples than did the sham-immunized monkeys (Fig. 6). The only exception was the group of unprotected, antigen III-immunized monkeys in which *S. mutans* almost reached the level found in the sham-immunized monkeys after 28 weeks. The cell-immunized monkeys showed consistently the lowest recovery of *S. mutans* throughout the experimental period. As the caries index in this group was comparable to that in the monkeys immunized with antigens I and I/II, the explanation for the lower level of *S. mutans* is not clear.

There can be little doubt that antigen I/II is highly immunogenic, in view of the finding that 0.1 mg in FIA or Alhydrogel induces a well-maintained level of antibodies. Antibodies could be detected by the radioimmunoassay at a dilution of 1:500,000, which suggests that they have a high avidity. It is also pertinent that 0.01 µg of antigen I/II per ml can induce T helper cell activity in lymphocytes from the same monkeys, thereby stimulating the development of antibody-forming cells (6).

It should be noted that no attempt was made in this investigation to find the optimal conditions for immunization. However, now that a protein antigen that has protective properties comparable to those of the whole cells of *S. mutans* has been isolated, further work will be required to define the optimum preparation, dose, adjuvant, and frequency of immunization.

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